

(19) World Intellectual Property
Organization
International Bureau



10/549619



(43) International Publication Date
30 September 2004 (30.09.2004)

PCT

(10) International Publication Number
WO 2004/083427 A3

(51) International Patent Classification⁷: C12N 15/10,
C12P 21/02

(21) International Application Number:
PCT/DK2004/000195

(22) International Filing Date: 22 March 2004 (22.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 2003 00430 20 March 2003 (20.03.2003) DK
60/455,858 20 March 2003 (20.03.2003) US

(71) Applicant (for all designated States except US): NUEVO-
LUTION A/S [DK/DK]; Rønnegade 8, 5., DK-2100
Copenhagen Ø. (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): FRANCH, Thomas

[DK/DK]; Humlebæksgade 14, st.tv., DK-2200 Copen-
hagen N (DK). JACOBSEN, Soeren, Nyboe [DK/DK];
Frederiksvej 36, 4.tv., DK-2000 Frederiksberg (DK).
RASMUSSEN, Torben [DK/DK]; Skovvej 13, DK-2750
Ballerup (DK). NEVE, Soeren [DK/DK]; Ellevaenget 4,
DK-2800 Lyngby (DK). PEDERSEN, Henrik [DK/DK];
Frodesvej 24, DK-2880 Bagsvaerd (DK). GOULIAEV,
Alex, Haahr [DK/DK]; Broendsted 223, DK-3670 Veksø
Sjælland (DK).

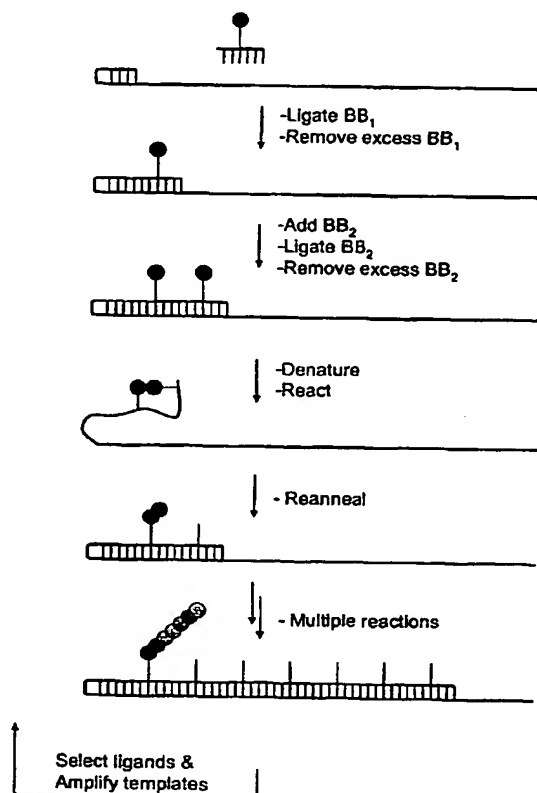
(74) Agent: HØIBERG A/S; St. Kongensgade 59A, DK-1264
Copenhagen K (DK).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,

[Continued on next page]

(54) Title: LIGATIONAL ENCODING OF SMALL MOLECULES

Synthesis of a linear molecule



(57) Abstract: The invention relates to a method for synthesising a bifunctional complex comprising an encoded molecule and an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the encoded molecule, said method comprising the steps of i) providing a) at least one template comprising one or more codons capable of hybridising to an anti-codon, wherein said template is optionally associated with one or more chemical entities, and b) a plurality of building blocks each comprising an anti-codon associated with one or more chemical entities, and ii) hybridising the anti-codon of one or more of the provided building blocks to the template, iii) covalently linking said anti-codons and/or linking the at least one template with the anti-codon of at least one building block, thereby generating an identifier polynucleotide capable of identifying chemical entities having participated in the synthesis of the encoded molecule, iv) separating the template from one or more of the anti-codons hybridised thereto, thereby generating an at least partly single stranded identifier polynucleotide associated with a plurality of chemical entities, v) generating a bifunctional complex comprising an encoded molecule and an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the encoded molecule, wherein said encoded molecule is generated by reacting at least two of said plurality of chemical entities associated with the identifier polynucleotide, and wherein said at least two chemical entities are provided by separate building blocks.



PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ,*

TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

Published:

- *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

- (88) **Date of publication of the international search report:**

17 February 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK2004/000195

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12P21/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CAB Data, Sequence Search, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2004/013070 A (NUEVOLUTION AS ; PEDERSEN HENRIK (DK)) 12 February 2004 (2004-02-12) page 69, line 18 - page 71, line 7; figures 19,23B page 75, line 22 - page 76, line 4 -----	1-123, 133-136, 153-198, 200-204
P,X	WO 2004/016767 A (HARVARD COLLEGE) 26 February 2004 (2004-02-26) cited in the application claims 1-103; figures 9,30-32,52-54,59,61 ----- -/--	1-123, 133-136, 153-198, 200-204

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier document but published on or after the international filing date
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
 "&" document member of the same patent family

Date of the actual completion of the international search

17 September 2004

Date of mailing of the international search report

27 DEC 2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hornig, H

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/DK2004/000195

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/103008 A (GUILAEV ALEX HAAHR ; NOERREGAARD-MADSEN MADS (DK); SLOEK FRANK ABILGA) 27 December 2002 (2002-12-27) cited in the application claims 1,77,78,104-132; figures 5-7 -----	1-123, 133-136, 153-198, 200-204
A	WO 02/074929 A (KANAN MATTEW W; GARTNER ZEV J ; LIU DAVID R (US); HARVARD COLLEGE (US)) 26 September 2002 (2002-09-26) cited in the application the whole document -----	1-123, 133-136, 153-198, 200-204
A	WO 95/04160 A (ISIS INNOVATION ; SOUTHERN EDWIN (GB); CUMMINS WILLIAM JONATHAN (GB)) 9 February 1995 (1995-02-09) claims 1-27; figure 4 -----	1-123, 133-136, 153-198, 200-204
A	WO 00/23458 A (UNIV LELAND STANFORD JUNIOR) 27 April 2000 (2000-04-27) cited in the application the whole document -----	1-123, 133-136, 153-198, 200-204
A	WALDER J A ET AL: "COMPLEMENTARY CARRIER PEPTIDE SYNTHESIS: GENERAL STRATEGY AND IMPLICATIONS FOR PREBIOTIC ORIGIN OF PEPTIDE SYNTHESIS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 76, no. 1, January 1979 (1979-01), pages 51-55, XP000857351 ISSN: 0027-8424 the whole document -----	1-123, 133-136, 153-198, 200-204
A	VISSCHER J ET AL: "TEMPLATE-DIRECTED SYNTHESIS OF ACYCLIC OLIGONUCLEOTIDE ANALOGUES" JOURNAL OF MOLECULAR EVOLUTION, SPRINGER VERLAG, NEW YORK, NY, US, vol. 28, no. 1/2, 1988, pages 3-6, XP000857353 ISSN: 0022-2844 the whole document -----	1-123, 133-136, 153-198, 200-204
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2004/000195

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-123 complete, 133-136, 153-198, 200-204 partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-123)-complete, (133-136,153-198,200-204)-partially

A method for synthesising a bifunctional complex comprising an encoded molecule and an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the encoded molecule, said method comprising the steps of (i) providing a) at least one template comprising one or more codons capable of hybridising to an anti-codon, wherein said template is optionally associated with one or more chemical entities, and b) a plurality of building blocks each comprising an anti-codon associated with one or more chemical entities, and (ii) hybridising the anti-codon of one or more of the provided building blocks to the template, (iii) covalently linking said anti-codon and/or linking the at least one template with the anti-codon of at least one building block, thereby generating an identifier polynucleotide capable of identifying chemical entities having participated in the synthesis of the encoded molecule, (iv) separating the template from one or more of the anti-codon hybridised thereto, thereby generating an at least partly single stranded identifier polynucleotide associated with a plurality of chemical entities, (v) generating a bifunctional complex comprising an encoded molecule and an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the encoded molecule, wherein said encoded molecule is generated by reacting at least two of said plurality of chemical entities associated with the identifier polynucleotide, wherein said at least two chemical entities are provided by separate building blocks;

2. claims: (124-129)-complete, (132-136,153-198,200-204)-partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method of synthesising one or more bifunctional complexes each comprising a molecule resulting from the reaction of a plurality of chemical entities and an identifier polynucleotide identifying one or more of the chemical entities having participated in the synthesis of the molecule, said method comprising the steps of (i) providing a plurality of building blocks each comprising an oligonucleotide associated with one or more chemical entities, (ii) providing at least one connector oligonucleotide capable of hybridising with one or more building block oligonucleotides, (iii) immobilising at least one building block to a solid support, (iv) hybridising said immobilized building block oligonucleotide to a first connector oligonucleotide, (v) hybridising at least one additional building block oligonucleotide to said first connector oligonucleotide, (vi) ligating building block oligonucleotides hybridised to the connector oligonucleotide, (vii) separating the connector polynucleotide from the ligated building block oligonucleotides, (viii) reacting one or more chemical entities associated with different building block oligonucleotides, thereby obtaining a first bifunctional complex comprising a first molecule or first molecule precursor linked to a first identifier oligonucleotide identifying the chemical entities having participated in the synthesis of the molecule or molecule precursor, wherein said first bifunctional complex is immobilized to a solid support;

3. claims: (130-131)-complete, (132-136,153-198,200-204)-partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method for synthesising a bifunctional complex comprising a molecule resulting from the reaction of a plurality of chemical entities, wherein said molecule is linked to an identifier polynucleotide identifying one or more of the chemical entities having participated in the synthesis of the molecule, said method comprising the steps of (i) providing a plurality of building blocks selected from the group consisting of a) building blocks comprising an identifier oligonucleotide linked to one or more chemical entities, b) building blocks comprising an identifier oligonucleotide linked to one or more reactive groups, c) building blocks comprising an identifier oligonucleotide comprising a spacer region, wherein said building blocks comprising a spacer region are preferably connector polynucleotides to which building blocks of group a) and b) can hybridise, (ii) generating a hybridisation complex comprising at least n building blocks by hybridising the identifier oligonucleotide one building block to the identifier oligonucleotide of at least one other building block, wherein n is an integer of 4 or more, wherein at least 3 of said at least n building blocks comprise a chemical entity, wherein no single identifier oligonucleotide is hybridised to all of the remaining identifier oligonucleotides, wherein optionally at least one of said building blocks of group c) is immobilised to a solid support, thereby providing a handle to which an oligonucleotide of at least one building block of groups a) or b) can hybridise, (iii) covalently linking identifier oligonucleotides of building blocks comprising one or more chemical entities, thereby obtaining an identifier polynucleotide comprising covalently linked chemical identifier oligonucleotides each associated with one or more chemical entities, (iv) optionally separating said identifier polynucleotide obtained in step (iv), from any immobilized connector oligonucleotide hybridised thereto, wherein said separation optionally comprises the step of diverting said identifier polynucleotide comprising covalently linked identifier to a different reaction compartment, thereby separating said identifier polynucleotide from said immobilised connector oligonucleotides, (v) reacting said at least 3 chemical entities linked to the identifier polynucleotide, and (vi) obtaining a bifunctional complex comprising a molecule resulting from the reaction of a plurality of chemical entities.

4. claims: (137-152,205)-complete,
(133-136,153-198,200-205)-partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method for synthesising a bifunctional complex comprising an encoded molecule and a template coding for one or more chemical entities which have participated in the synthesis of the encoded molecule, the method comprising the steps of (i) providing a) a template comprising one or more codons, b) one or more building blocks having an anti-codon associated with a chemical entity, and c) a nucleic acid sequence associated with a reactive site, (ii) contacting the template with one or more building blocks under conditions allowing for hybridisation between codon and anti-codons, (iii) ligating at least one anticodon of a building block to the nucleic acid sequence associated with the reactive site, and (iv) reacting the chemical entity of the ligated building block with the reactive site under conditions where the ligation product is single stranded, to obtain a template-encoded reaction product;

5. claim: 199

A library of different complexes, each complex comprising an encoded molecule and a template, which has encoded the chemical entities which has participated in the synthesis thereof, said library being obtainable by processing a plurality of building blocks;

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK2004/000195

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